18 ~~23~~. The method of Claim ~~22~~ 17, wherein the influenza virus antigen is hemagglutinin.

19 ~~24~~. The method of Claim ~~23~~ 18, wherein the hemagglutinin is subtype H1 or H7.

Remarks

Claim Status

Claims 1-18 were pending. Claim 3 has been cancelled, and Claim 4 has been amended to depend from Claim 1. Claims 5 and 15 have been cancelled and rewritten as independent claims 19 and 22. Claims 6 and 16 have been cancelled and rewritten as Claims 20 and 23. Claims 21 and 24 have been added. Support for these claims is found throughout the Specification; no new matter has been added.

Rejection of Claims under 35 U.S.C. 112, first paragraph

The Examiner maintained the rejection of claims 1-18, stating that:

> the disclosure is enabling only for claims limited to a method of immunizing [a] vertebrate by administering a DNA transcription unit encoding H1 and H7 influenza hemagglutinin antigens in nonhuman animals....

Applicants respectfully disagree with this assessment. To facilitate prosecution of the application, Claims 5 and 15 have been cancelled and rewritten as independent claims directed to methods of immunizing a vertebrate against influenza virus by administering to a vertebrate a DNA transcription unit comprising an influenza virus antigen.

As described in the Specification, Applicants have described protection against both subtype H1 and H7 influenza hemagglutinin antigens. These subtypes provide models for other hemagglutinin subtypes. One of ordinary skill in the art, using the Specification, would be able to utilize transcription units comprising DNA encoding

hemagglutinin antigens of other subtypes.

Furthermore, as described in the Specification, both chickens and mice have been shown by Applicants to be protected against influenza virus as a result of immunization using Applicants' claimed invention. Also, as described in the Declaration of Harriet L. Robinson which was submitted as Exhibit B with an Amendment filed on February 22, 1994, ferrets have also been shown to be protected against influenza virus. Thus, Applicants have shown successful immunization of a range of vertebrate species. Furthermore, these animal models are reasonably predictive of successful immunization in humans. For example, ferrets are a recognized host for experimental influenza virus infections, because they are permissive hosts for human influenza viruses (see Fields, ed., Virology, 2nd Edition (1990), Volume I, pp. 1114-1115; copies of these pages are attached as Exhibit 1). Therefore, Applicants respectfully submit that the Specification enables one of ordinary skill in the art to immunize any vertebrate animal, including humans, against influenza virus.

In addition, Applicants respectfully submit that influenza virus is representative of the pathogens for which immunization can be achieved utilizing the methods of the current invention. One of ordinary skill in the art, using the Specification, would be able to utilize transcription units comprising DNA encoding other desired antigens.

Rejection of Claims under 35 U.S.C. 103

The Examiner maintained the rejection of Claims 1-4 as being unpatentable over King. The Examiner indicated that:

> Applicant urges that King describes injection of a construct containing the gene for gp120 for the production of cytotoxic T cells in mice and that mice do not develop AIDS upon being infected with HIV and

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> that this model cannot be used to test for protective immunization. It is the Examiner's position that King states that this is a novel technique[ ] because it uses naked plasmid DNA or mRNA to produce the MHC class I restricted cytotoxic T cell response. Therefore one would reasonably expect this novel technique to be effective in generating cellular as well as cell-mediated immune responses against HIV that would be protective.

Applicants respectfully disagree with this assessment.

Applicants note that Claim 3 has been cancelled, and therefore, the rejection is moot as to it. Claims 1 and 2 pertain to a method of immunizing a vertebrate animal against an infectious agent by administering a DNA transcription unit that includes DNA encoding an antigen linked to a promoter region, thereby eliciting a humoral and/or cell-mediated immune response, and whereby the animal is protected against disease caused by the infectious agent.

The King reference describes injection of a construct containing the gene for gp-120, a cytomegalovirus (CMV) early promoter sequence and tissue plasminogen activator (TPA) sequence, into the muscle of a mouse, and the resultant production of cytotoxic T cells in the mice against gp-120 protein. King does not teach or describe any data demonstrating that inoculation with a gene for a particular epitope of influenza would prevent infection *in vivo*; nor does King provide any data demonstrating that antigenic protection can be achieved against <u>any</u> disease upon challenge.

One of ordinary skill in the art would not expect that injection of a construct, as described by King, and the resultant production of cytotoxic T cells in the mice, would lead to protection against disease. Cytotoxic T lymphocyte response to an antigen is not necessarily indicative of protection. For example, cytotoxic T lymphocytes are not a necessary component for protective

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immunizations against influenza virus in the murine model (see Scherle, P.A. *et al.*, *J. Immunol. 148*:212-217 (1992), attached as Exhibit 2; Eichelberger, M. *et al.*, *J. Exp. Med. 174*:875 (1991), attached as Exhibit 3). Furthermore, in the chicken influenza virus model, cytotoxic T lymphocyte responses have not provided protection (Brown, D. W. *et al.*, *Avian Diseases 36*:515-520 (1992), attached as Exhibit 4). Thus, it is known in the art that production of cytotoxic T cells does not necessarily correlate with protection upon challenge. Therefore, one of ordinary skill in the art would not have had a reasonable expectation of success in generating a protective immune response.

Rejection of Claims under 35 U.S.C 103

The Examiner maintained the rejection of Claims 5-18 as being unpatentable over WO 90/11092 in view of Huylebroeck et al. The Examiner stated that:

> Given the concern and focus in the art on effective vaccines against influenza and given the well known fact that the major response to influenza infection is directed to the immunodominant hemagglutinin molecule, one would be motivated, by these two well known facts alone, to include, in a DNA transcription unit (taught by Huylebroeck et al) the gene for the hemagglutinin molecule as part of a method of delivering polynucleotides into a cell (as taught by WO 90/11092) with the expectation of generating protective immune responses against influenza.

Applicants respectfully disagree with this assessment. The claims of the current invention would not have been rendered obvious by the combination of WO 90/11092 with Huylebroeck et al. for several reasons: the vector and protein production methods described by Huylebroeck et al. differ significantly from those of the current invention, such that one of ordinary skill would not have been

motivated to combine WO 90/11092 with Huylebroeck et al.; furthermore, there is no teaching or suggestion supporting the combination. Even if the references were combined, one of ordinary skill would not have had a reasonable expectation of successfully achieving the claimed results, because of the differences in amount of antigen that are needed to vaccinate by protein inoculations and the amount of antigen produced by direct DNA inoculation.

Applicants note that Claims 5 and 15 have been cancelled and rewritten as independent claims. The Claims, as amended, pertain to a method of immunizing a vertebrate, such as a mammal, against an infectious agent, such as influenza virus, by administering a DNA transcription unit including an antigen, such as an influenza virus antigen, linked to a promoter; eliciting a humoral and/or cell-mediated immune response, or both; and thereby protecting the vertebrate against disease.

WO 90/11092 describes methods of delivering RNA or DNA polynucleotides into a vertebrate cell by interstitial delivery, exemplified by mRNA vaccination of mice to produce gp120 of HIV. The level of protein produced by delivery of polynucleotides was less than 400 picograms, as shown in Figure 3 and described in Examples 12 and 13.

Huylebroeck et al. describe use in cell culture of an SV40 late replacement vector to produce HA to be used in vaccination. This vector is designed to undergo episomal replication in eukaryotic cells. It includes the complete early region of the SV40 genome, as well as the SV40 origin of replication. The late region of the SV40 genome is replaced by a polylinker site that allows cloning of genes in the position for SV40 structural proteins. The expression of the SV40 early region plus the SV40 origin of replication supports episomal replication and amplification of the vector DNA in eukaryotic cells, to enhance levels of protein produced by the vector in the cell culture.

Huylebroeck et al. do not teach or suggest that influenza hemagglutinin could be generated in vivo in an animal by DNA inoculation, as in the current invention. Huylebroeck et al. teach only in vitro culture systems. There is no teaching or suggestion to introduce the vectors in vivo.

In sharp contrast, in the current invention, DNA encoding only the particular antigens, such as hemagglutinin, is introduced in vivo, in order to produce protein in an animal. The DNA used in the invention does not use SV40 sequences; does not encode a replication-competent vector; and is not capable of replication in the host. The early region of the SV40 genome which supports episomal replication of DNA is the oncogenic region of SV40; the tumor antigens encoded by this region support transformation of cells and tumor induction. Such tumor inducing genes are inappropriate for introduction in vivo.

In order for references to be combined, there must be some teaching or suggestion in the prior art of record supporting the combination (ACS Hospital Systems, Inc. v. Montefiore Hospital, 221 USPQ 929, 933 (CAFC 1984)). However, one of ordinary skill in the art would not have been motivated to look beyond the general teachings of WO 90/11092 concerning delivery of polynucleotides, to the teachings of Huylebroeck et al. concerning production of influenza virus hemagglutinin in cell culture. There is no teaching or suggestion in WO 90/11092 that one of ordinary skill should look to the Huylebroeck et al. reference, which teaches influenza virus in particular, as opposed to looking to a reference describing any other possible viruses or pathogens. WO 90/11092 mentions muscular dystrophy, cystic fibrosis, genetic defects of intermediary metabolism, HIV, Alzheimer's disease, liver and lung disease caused by alpha-1-antitrypsin deficiency, cancers, and controlled release of therapeutic peptides, for example, but does not mention influenza at all.

Furthermore, even if one were motivated by "the concern and focus in the art on effective vaccines against influenza and given the well known fact that the major response to influenza infection is directed to the immunodominant hemagglutinin molecule", one of ordinary skill in the art would not have been motivated to combine WO 90/11092 with Huylebroeck et al.. The Examiner states that one of ordinary skill in the art would have been motivated to combine "in a DNA transcription unit (taught by Huylebroeck et al) the gene for the hemagglutinin molecule as part of a method of delivering polynucleotides into a cell (as taught by WO 90/11092)". However, as described above, there is no mention at all in WO 90/11092 of influenza virus; furthermore, Huylebroeck et al. teach protein production in cell culture, and not in an animal; and the vector used by Huylebroeck et al. is significantly different from that used in the current invention and would be inappropriate for use in vivo. Therefore, one would not have been motivated to combine the teachings of Huylebroeck et al. regarding protein production in cell culture using a replication competent vector with the method of delivering polynucleotides described in WO 90/11092.

In addition, obviousness is established only if the teachings of the cited art would suggest the claimed invention to one of ordinary skill in the art with a reasonable degree of certainty of successfully achieving the claimed results. One of ordinary skill in the art would not have had a reasonable expectation of success in achieving the claimed results. One of ordinary skill in the art would not have had a reasonable expectation that utilization of DNA encoding a particular antigen, such as hemagglutinin, would result in protection of vertebrate animals against infection and disease, such as influenza. As described above, an immune response such as that described in WO 92/11092 is not necessarily indicative of

the ability of the vaccine to protect against infection. Furthermore, one of ordinary skill in the art seeking to provide protective immunization would have expected that microgram quantities (generally 10 to 100 micrograms) of protein would have been needed (see Fields, Orthomyxoviruses Vol. I, pp. 1126-1127, concerning the amounts of protein used in inactivated influenza virus vaccines to obtain protection; a copy of this reference was attached as Exhibit B to the Amendment filed on February 22, 1994). Only picogram levels of protein expression were achieved through delivery of polynucleotides as described in WO 90/11092 (see Figure 3). One of ordinary skill in the art would not have thought that these minute levels of protein expression could achieve protective immunizations. Thus, one of ordinary skill in the art would have been discouraged from attempting to generate protective immunization using the methods described in WO 90/11092, because of the small amounts of protein produced. Furthermore, WO 90/11092 teaches expression of HIV gp120 protein in mice. Because mice cannot be infected with HIV, it would not have been possible in WO 90/11092 to demonstrate protective immunization. Applicants have, for the first time, shown that inoculation by administering a DNA transcription unit encoding a desired antigen, such as hemagglutinin, results in protection from disease caused by an infectious agent.

Conclusion

In view of the amendments and the arguments presented above, Applicants respectfully submit that the Claims are in condition for allowance, and request that the Examiner reconsider and withdraw all rejections.

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If the Examiner believes that a telephone conversation will expedite prosecution of this application, the Examiner is requested to call Applicants' Attorney at (617) 861-6240.

Respectfully submitted,

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